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PHARMACOKINETIC STUDIES OF CEFAZOLIN IN HORSES AND CATTLE

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I. EXECUTIVE SUMMARY

ABSTRACT

Pharmacokinetic studies of the antibiotic cefazolin were undertaken to determine the feasibility of prolonging plasma drug concentrations by administering a sustained-release form of the drug. Benzathine-cefazolin (DBED-CZ) was synthesized in our laboratory and tested for stability. DBED-CZ was injected into horses and cattle and comparisons were made by injecting cefazolin-sodium intravenously (IV) and intramuscularly (IM) in a cross-over study. An assay was developed using high pressure liquid chromatography (HPLC) and plasma concentrations measured following each injection. In horses and cattle, drug concentrations persisted for 3 to 8 hours for IV and IM sodium cefazolin respectively, but were detected for at least 24 hours following IM DBED-CZ. The prolonged plasma levels achieved were due to a slow, but incomplete absorption of the drug from the intramuscular site.

LAY SUMMARY

Cephalosporin antibiotics are a valuable group of antibiotics in veterinary medicine for dogs and cats because they have greater activity against many bacteria than traditional antibiotics (eg, penicillin, sulfonamides) and are associated with fewer side effects in comparison with many other antibiotics (eg, chloramphenicol, aminoglycosides). The use of cephalosporins in large animal medicine has been limited however, because cephalosporins are eliminated from the body very rapidly, usually with a half-life of less than one hour, which necessitates frequent and expensive dosing.

We have undertaken experiments to examine the feasibility of synthesizing an insoluble, sustained-release form of the cephalosporin, cefazolin and injecting it intramuscularly (IM) into horses and cattle. An insoluble form of cefazolin would presumably be absorbed much more slowly from an intramuscular injection site resulting in

antibiotic plasma concentrations that persist longer than currently available formulations of the drug. Sustained-release cefazolin was synthesized in our laboratory by conjugating cefazolin with dibenzylethylenediamine (benzathine, DBED) to form a poorly-soluble salt of the drug. Our assays show this salt to be stable for at least six months, and retain the chemical properties of the parent drug, cefazolin.

The plasma concentration-time profile of the newly-synthesized cephalosporin salt was measured by injecting it intramuscularly (IM) in horses and cattle and subsequently measuring plasma concentrations of the drug and comparing the plasma level attained with DBED-cefazolin with those attained from intravenous (IV) and IM injections of equal doses of a commercially-available sodium salt of cefazolin. We developed an assay for the drug in plasma using a sensitive and specific chromatographic method, high pressure liquid chromatography (HPLC).

The results from our studies indicate that antibiotic drug concentrations of DBED-cefazolin persist much longer than either the IV or IM injections of the water-soluble form of sodium-cefazolin. Cefazolin falls below therapeutic drug levels in 3 hours and 6 to 8 hours for IV and IM administration, respectively of sodium cefazolin, whereas cefazolin plasma concentrations, although lower, persist for 24 hours following an injection of an equal dose of DBED-cefazolin.

It is not known what the future impact will be for a sustained-release form of cefazolin. These results indicate that it is possible to achieve persistent drug levels for as long as 24 hours from DBED-cefazolin. The commercial use of DBED-cefazolin may allow one to administer the drug only once per day and thus achieve greater compliance with lower overall cost and less stress to some animal patients.

II. TECHNICAL REPORT

ABSTRACT

Pharmacokinetic studies of the cephalosporin antibiotic cefazolin in horses and cattle were undertaken to determine the feasibility of prolonging the plasma drug concentrations by administering a sustained-release form of the drug. A form of benzathine-cefazolin, dibenzylethylenediamine-cefazolin (DBED-cefazolin) was synthesized in our laboratory and tested for stability. It is a poorly soluble salt and is released from an intramuscular injection slowly, thus prolonging the duration of drug plasma concentrations. DBED-CZ was injected into six horses and six cattle and cross-over comparisons were made with a water-soluble, commercially available form of cefazolin (cefazolin-sodium) injected IV and IM. A drug assay was developed using reverse phase high pressure liquid chromatography (HPLC) and plasma concentrations were measured following each injection until the drug was eliminated.

In horses, plasma concentrations attained a mean peak of 226.03 (± 89.55*) mcg/ml and declined rapidly with an elimination half-life of 33.6 min. Plasma concentrations remained above 1.0 mcg/ml for only three hours. Following IM administration of cefazolin sodium, plasma concentrations in horses attained a peak of 34.4 (± 13.17) mcg/ml and remained above 1.0 mcg/ml for approximately 6 to 8 hours. Plasma concentrations declined with an elimination half-life of 1.26 hours. Following IM administration of DBED-cefazolin, plasma concentrations attained a peak of 3.48 (± 0.61) and remained above 1.0 mcg/ml for 24 hours.

In cattle, plasma concentrations attained a mean peak of 237.29 (± 85.32) mcg/ml and declined rapidly with an elimination half-life of 31.5 min. Plasma concentrations

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* Values listed are ± one standard deviation.

remained above 1.0 mcg/ml for 4.5 hours. Following IM administration of cefazolin sodium, plasma concentrations in horses attained a peak of 30.46 (± 13.5) mcg/ml and remained above 1.0 mcg/ml for approximately 6 to 8 hours. Plasma concentrations declined with an elimination half-life of 1.41 hours. Following IM administration of DBED-cefazolin, plasma concentrations attained a peak of 4.06 (± 3.17) mcg/ml and remained above 1.0 mcg/ml for 24 hours.

The animals did not experience any adverse effects from DBED-cefazolin. The prolonged plasma levels achieved were due to a slow, but incomplete absorption of the drug from the intramuscular site. This preliminary study suggests that it may be possible to administer a sustained-release form of cefazolin (DBED-cefazolin) once per day, and avoid the labour and expense of the currently recommended dosage schedule for cefazolin of 4 to 6 times per day. Further work should be carried out, however to determine if the drug levels attained with DBED-cefazolin are sufficiently high to eradicate bacteria in animals with a clinical infection.

BACKGROUND

The cephalosporin antibiotics are a tremendously valuable group of antimicrobials and have experienced extensive use in people, dogs, and cats. There has been rapid growth in their development as more than 30 cephalosporins are available in human medicine. They are remarkably safe and are effective against a broad spectrum of both gram-positive and gram-negative organisms encountered in bovine and equine medicine, including E.coli, Klebsiella spp., penicillin-resistant staphylococci, penicillin-resistant Pasteurella haemolytica, Corynebacterium spp., and streptococci (Papich, 1987; Papich, 1984; Thomson, et al., 1984, Sams, et al., 1985). Of the injectable cephalosporins, cefazolin is the most commonly used in human medicine (Abramowicz, 1983; Spector, 1986) and it is also a common choice in small animals. It is reported to be more active

against gram-negative bacteria (eg, E. coli, Salmonella spp., and Klebsiella spp.) than other first-generation cephalosporins (Thompson and Wright, 1983; Caprile, 1988; Abramowicz, 1985). Cefazolin is the preferred antibiotic for treating certain bone and joint infections in people (Cunha, 1977; Schurman, et al. 1978; Fitzgerald and Thompson, 1983; Dickie, 1986; Daly, et al., 1982; Abramowicz, 1983), and it is the preferred antibiotic for administration prior to surgery, including orthopedic and abdominal surgery (Abramowicz, 1985; Kaiser, 1986; Spector, 1986; Nelson, 1982). Its advantages include a broad spectrum of antimicrobial activity good tissue levels and it produces less pain following IM injection than other cephalosporins.

Despite its potential efficacy against many equine bacterial pathogens, cefazolin has not been administered commonly to horses because the elimination of cefazolin is very rapid with an elimination half-life of 35-46 minutes following IV administration (Sams and Ruoff, 1985), which necessitates frequent dosing. Other cephalosporins are eliminated from horses even more quickly (Caprile, 1988). The pharmacokinetics have not been reported for cattle. Because prolonged antibiotic concentrations of cefazolin would be desirable, Donecker, et al. (1986) tested the effects of probenecid for prolonging the plasma concentrations of cefazolin in mares. Although probenecid will decrease the renal excretion of other beta-lactam antibiotics (eg. penicillin G) and prolong the plasma concentrations, it did not prolong the elimination of cefazolin in horses. There are no commercially-available sustained release formulations of cephalosporin antibiotics as there are for penicillin G (eg, procaine-penicillin, benzathine-penicillin) which, owing to their slow absorption from an intramuscular depot, prolong the plasma drug concentrations of penicillin and decrease the frequency of injection. Guerrini et al (1986) attempted to produce prolonged plasma concentrations of a third-generation cephalosporin, cefotaxime by administering it in an oily suspension to dogs and sheep. After an IM injection in

sheep the oily suspension only slightly prolonged the plasma concentration as it was above 1.0 mcg/ml for 6.5 hours following an injection of the oily suspension, and 4.3 hours following an injection of the aqueous formulation. The oily suspension had no effect on the elimination half-life in dogs, but it slightly prolonged the elimination half-life after a subcutaneous injection in sheep. There is no commercial preparation of a oily suspension of any cephalosporins currently available.

Because there are no prolonged release formulations of a cephalosporin antibiotic available for intramuscular use, this study was undertaken to examine the feasibility of synthesizing such a preparation and administering it to horses and cattle.

In order to evaluate the absorption characteristics and elimination of a newly synthesized formulation of cefazolin (DBED-cefazolin) it is necessary to perform a cross-over study. A crossover study allows one to compare the pharmacokinetics of cefazolin sodium following an IV and IM injection with the pharmacokinetics following an IM injection of the newly-synthesized formulation. A cross-over design is advantageous because the plasma concentrations and elimination rates can be compared using each animal as its own control and minimizes the need for large numbers of experimental animals.

OBJECTIVES

1. Synthesize an insoluble salt of cefazolin suitable for intramuscular injection in horses and cattle [dibenzylethylenediamine (DBED)-cefazolin].

2. Synthesize a procaine-cefazolin salt suitable for intramuscular injection in horses and cattle.

3. Confirm the structure of the synthesized product by NMR spectra.

4. Develop an assay method for measuring cefazolin concentrations in plasma by high pressure liquid chromatography (HPLC).

5. Confirm the stability of the synthesized cefazolin product by HPLC analysis.

6. Measure the disposition, elimination, clearance, systemic availability, and duration of plasma levels of cefazolin following intravenous (IV) injections of cefazolin-sodium, intramuscular injections (IM) of cefazolin-sodium, and IM injections of DBED-cefazolin and procaine-cefazolin.

EXPERIMENTAL METHODS

Synthesis of DBED-Cefazolin:

The procedure for the synthesis of dibenzylethylenediamine (benzathine), (DBED)-cefazolin was as follows: DBED was converted to its diacetate salt with acetic acid. The sodium salt of cefazolin (Na-cefazolin) was mixed with DBED-acetate in aqueous solution (0.49 ml, 4.1 mmoles) by dissolving DBED-acetate in water and adding slowly with stirring the sodium salt of cefazolin (2 g, 4.2 mmoles) in aqueous solution at 25° C. The suspension was stored at 4° C for one hour and the solid was recovered by filtration under vacuum to give crude DBED-cefazolin (yield, 90 %, m.p. 143-144°). Crystallization from aqueous acetone gave an analytically pure sample of DBED-cefazolin ($C_{44}H_{48}N_{12}O_8S_6$; yield 75 %); m.p. 163-164°; lambda maximum (pH 7.0 272 nm (extinction = 26,500) and lambda minimum of 231 mn (extinction = 16,600). Approximately 150 grams of DBED-cefazolin was synthesized. Each batch synthesized for injection into animals met the following criteria: m.p. 143-145° C; U.V. (phosphate buffer, pH 7.0) lambda $_{max}$ 272 nm

(extinction 26,850 +/- 500) and elementary analysis C,H,N for $C_{44}H_{48}N_{18}O_8S_6$ was +/- 0.5 of the calculated values. Typical analytical values found were: C, 45.95; H, 4.20; and N, 22.30. Calculated values are: C, 45.98; H, 4.21; and N, 21.94. All preparations were shown to be homogeneous by HPLC analysis. The structure of DBED-cefazolin has been also confirmed by NMR spectra.

Synthesis of Procaine-Cefazolin:

As reported in previous progress reports, after many attempts to synthesize procaine-cefazolin in the pure state, we were unsuccessful. Procaine-cefazolin was generated in situ, but it was very unstable and undergoes rapid deterioration in solution. Therefore, it was impossible for us to carry out the experiments listed in the objectives for procaine-cefazolin.

Stability Studies:

All DBED-cefazolin preparations were vacuum dried for 48 h at 20° and stored in a desiccator protected from light. Each sample was checked periodically for deterioration by HPLC (see procedure details below). When stored as a dry powder, no decomposition was observed for up to six months (maximum test period). In aqueous suspension, DBED-cefazolin was stable up to 72 hours at 4°. At the end of 4 days some decomposition occurred as suggested by the presence of additional peaks in HPLC profiles.

Analytical Procedure:

An analytical procedure using HPLC was standardized for the assay of cefazolin in biological fluids, stability studies, and quality control analysis of the finished product. This analysis method is simple, rapid, sensitive, requires relatively small sample volume, and thus is readily applicable for the analysis of cefazolin. Our methods were modified from the procedures of others that have been published (Brissom and Fourtillan, 1981; Sams and Ruoff, 1985).

The analysis consisted of a C_{18} reverse-phase column (u-Bondapak®-RCM: Waters Assoc.) (8 mm x 10 cm) at ambient temperature; mobile phase consisting of 0.01 M acetate buffer (pH 4.8): methanol (25%, V/V); inlet pressure, 500 psi, and flow rate of 1.5 ml/min. Cefamandole was incorporated as an internal standard to improve accuracy of the method. Cefazolin was detected at a wavelength of 272 nm using 0.100 absorbance units full scale on a U.V. detector. Retention times were: cefamandole, 13.1-13.5 min and cefazolin 6.5-6.8 min. Representative chromatograms are shown in Figure 1.

The liquid chromatography system (Waters Assoc.) consisted of: programmable solvent delivery module (Model 490), programmable multiwavelength detector (Model 490), data module (Model 745, set at an attenuation of 128) and manual sample injector (Rheodyne) with a 20 microliter loop was used.

The standard curves were generated by calculating the peak height ratio of cefazolin/cefamandole from the chromatogram and performing linear regression on peak height ratio vs cefazolin concentration. The standard curves for cefazolin in water, cefazolin in bovine plasma and cefazolin in equine plasma were linear with regression coefficients (R^2) of at least 0.998. The standard curves are shown in Figure 2 and 3. Standard curves of cefazolin spiked with cefamandole internal standard (10mcg/mL) were linear from 0.25 mcg/mL (lowest limit of detection) up to 250 mcg/mL (highest concentration used). If samples contained greater than 250 mcg/mL of cefazolin, they were diluted until the concentration fell within the limits of the standard curve.

The extraction of cefazolin from plasma was performed by pipetting 1.0 mL of the bovine or equine plasma sample into a centrifuge tube, 20 ul of cefamandole (1.0 mg/ml) was added, the mixture was vortexed for 20 sec and centrifuged (2,000 rpm) for 10 minutes. The supernatent was transferred to another centrifuge tube, dichloromethane (5.0 mL) was added, and the solution was thoroughly mixed. The aqueous phase was

removed, filtered using 0.45 um filter (to remove particulate matter) and injected into the HPLC system.

Administration of Drugs to Horses:

Six horses were used. Four were mares and two were geldings, weighing from 395 to 602 kg (Table 1,2, and 3). They were housed in the outdoor Animal Care Facilities of the Western College of Veterinary Medicine and allowed free choice hay and water. During the experiments they were kept in individual stalls in the Veterinary Teaching Hospital of the W.C.V.M. All horses received physical exams, hematology tests and a biochemistry profile prior to the study to ensure they were healthy. One horse was treated with a parasiticide (ivermectin) one week prior to the experiment.

The experimental drugs were administered to the horses in a randomized, cross-over design. All horses received cefazolin-sodium (Kefzol^{R}-Lilly) IV, IM and DBED-cefazolin on three occasions with a one-week washout period between each treatment. Thus, each horse received all three treatments on different occasions. The DBED-cefazolin was prepared for injection in our laboratory by mixing it carefully with sterile water to form a suspension of 100 mg/ml (of the DBED salt). Each drug was injected at an approximate dose of 20 mg/kg of the cefazolin base. All the doses are listed in Tables 1, 2, and 3. The IM drugs were injected at multiple sites in the gluteal and neck muscles. The IV drug was injected as a rapid IV bolus in the jugular vein on the contra-lateral side of the vein that was used for collection of blood samples. Following each injection the horses were observed carefully for signs of anaphylaxis or other adverse reactions.

All horses were catheterized on the day before injection with a 12 ga. jugular catheter to facilitate blood collection. The catheters were sutured in place and held secure with tape and elastic wrap. The catheter was kept from clotting by filling it with hepar-

inized saline between samples. After drug injection, 7 mL of blood was collected via the catheter and transferred to a heparinized collection tube. Samples were collected at 0, 3, 6, 10, 15, 20, 30, 45, and 60 min and 1.5, 2, 3, 4, 6, 8, 10 and 12 hr following the IV injection of cefazolin-sodium; 0 15, 30, 45, 60 min and 1.5, 2, 3, 4, 6, 8, 10, and 12 hr following the IM injection of cefazolin-sodium and 0, 15, 30, 45, and 60, min, and 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 30, 36, and 48 h following the IM injection of DBED-cefazolin. The samples were kept on ice until they were centrifuged to collect the plasma. All samples were kept at -20° C until they could be assayed.

Administration of Drugs to Cattle:

Six cattle were used. Two were females, and four were steers. One animal was a Red Angus and the others were Hereford. Their weights ranged from 333 to 556 kg. The cattle were kept on a farm south of the WCVM (operated by V.I.D.O.). They were fed a hay and grain ration and free choice water. All cattle received physical examinations, hematology tests, and a biochemistry profile prior to the study to ensure they were healthy. They were kept together in an outdoor pen throughout the experimental period.

The drugs were administered to the cattle in an identical fashion as for the horses. The collection times were also the same, except that the samples were collected at 0, 3, 5, 10, 15, 20, 30, 45, and 60 minutes, and 1.5, 2, 4, 6, 8, 10, and 12 hours following IV administration, and 0, 15, 30 45, and 60 minutes, and 1.5, 2, 4, 6, 8, 10, and 12 hours following administration of the sodium cefazolin IM, and DBED-cefazolin IM. When DBED-cefazolin was administered, additional samples were collected at 24, 32, 36, and 48 hours.

Data Analysis:

All data was first graphed on a semilogarathmic graph with log plasma concentration vs time. The IV data was determined to be bi-exponential (see Figures 4 and 12)

and was analyzed by performing a pharmacokinetic compartmental analysis according to methods described by Gibaldi and Perrier (1982). A computer program (SAAM-29) was used to perform non-linear analysis on the plasma vs time curves and arrive at values for alpha (α: distribution rate constant), beta (β: elimination rate constant), "A" (y-intercept of the distribution curve), and "B" (y-intercept of the elimination). For the IM data, the elimination rate (β) was calculated after the absorption phase was completed (ie, after the peak plasma concentration was identified). It was not possible to calculate the IM absorption rate constant because absorption was practically instantaneous (T_{MAX} was 0.42 and 0.5 hours for horses and cattle, respectively).

Absorption of cefazolin from the IM injection of DBED-cefazolin exhibited zero order absorption and an elimination rate could not be calculated because it was not possible to identify the point at which absorption was complete.

A fortran program available on the U. of S. VAX 8600 computer was used to calculate the area under the plasma concentration vs time curve (AUC) and the apparent volume of distribution at steady state (Vd_{ss}) for the curves from all three treatments. The following formulae were used to calculate other pharmacokinetic parameters:

A. half-life: $T\ 1/2 = \ln 2/\beta = 0.693 / \beta$

B. apparent volume of distribution:
V_{AREA} = dose/(β x AUC)

C. apparent specific volume of distribution:
$V'_D = V_{AREA}$ / (kg body weight)

D. total systemic clearance: Cl_s = dose/AUC

E. systemic availability (F):
AUC (for IM DBED or IM Na-Cefazolin)/AUC (for IV Na-Cefazolin)

F. volume of central compartment: V_c = dose / (A + B)

G. microdistribution rate constants:

1. $K_{21} = [(A \times beta) + (B \times alpha)]/(A+B)$
2. $K_{10} = (alpha \times beta) / K_{21}$
3. $K_{10} = alpha + beta - K_{21} - K_{10}$

Results: Equine Studies

The pharmacokinetic parameters measured for horses are listed on Tables 1,2, and 3 for IV sodium cefazolin administered IV and IM, and DBED-cefazolin administered IM, respectively. The corresponding plasma concentrations are listed in Tables 4, 5, and 6. Figure 5 represents the mean plasma concentrations and Figures 6 to 11 present the plasma concentrations for the individual animals and the mean plasma concentrations (± standard error of the mean) measured after each drug administration.

The elimination following IV administration could be described by a two compartment open pharmacokinetic model with first order elimination (Figure 4). Sodium cefazolin administered IV was characterized by a rapid distribution rate (α half-life: 6.78 minutes) and rapid elimination rate (β half-life: 33.6 minutes). Sodium cefazolin exhibited a small apparent specific volume of distribution ($V'd_{AREA}$) that was 0.28 l/kg (± 0.08) and the volume of the central compartment (V_c) was 0.09 l/kg (± 0.03). The systemic clearance was high at 350 ml/kg/hr (±70).

Elimination following an IM injection of sodium cefazolin, on the other hand exhibited a much lower peak plasma concentration but a more prolonged elimination (β half-life: 1.26 hours). It was not possible to calculate the absorption rate constant or to perform a compartmental analysis because the absorption was rapid (T_{MAX}: 25 minutes). Absorption was complete and exhibited an apparent bioavailability that was greater than 100 percent.

Absorption of the DBED-cefazolin was slow and incomplete. The peak plasma concentration (C_{MAX}) was 3.48 mcg/ml (± 0.61) in comparison to 34.4 mcg/ml (± 13.17) for sodium cefazolin administered IM. Although plasma concentrations were low, cefazolin mean concentrations remained above 2 mcg/ml for 12 hours and above 1 mcg/ml for 24 hours (Figure 5) following the injection of DBED-cefazolin.

There was no pain or other adverse effects associated with the injection of the newly synthesized DBED salt.

Results: Bovine Studies

The pharmacokinetic parameters measured are listed on Tables 7, 8, and 9 for the IV sodium cefazolin administered IV, sodium cefazolin administered IM, and DBED-cefazolin administered IM, respectively. The corresponding plasma concentrations are listed in Tables 4, 5, and 6 and are represented graphically for the means in Figure 13 and for the individual animals in Figures 14 through 19.

The elimination following IV administration could be described by a two compartment open pharmacokinetic model (Figure 12) with first order elimination. As with the equine studies, elimination was characterized by a rapid distribution (α half-life: 7.8 min) and rapid elimination (β half-life: 31.5 min).

Sodium cefazolin exhibited a small apparent specific volume of distribution ($V'd_{AREA}$) in cattle (230 mg/kg ± 130) and the volume of the central compartment was similar to horses (90 ml/kg ± 30). Other parameters were also very similar to what was reported for horses.

Elimination following an IM injection of sodium cefazolin, was more prolonged than the IV administration. Although the peak plasma concentration was lower (C_{MAX}) at 30.46 mcg/ml (± 13.5), the elimination half-live (β half-life) was 1.41 hours. Absorption was rapid and complete with a systemic availability (F) of 100.26 percent. As with the

equine studies, it was not possible to calculate an absorption rate constant, or perform a compartmental analysis of cefazolin administered IM.

As with the equine studies, absorption of cefazolin from an injection of DBED-cefazolin was slow and incomplete. The peak plasma concentration (C_{MAX}) was 4.06 mcg/ml (± 3.17) in comparison to 30.46 mcg/ml (± 13.5) for sodium cefazolin administered IM. Following the injection of DBED-cefazolin, mean plasma concentrations remained above 2 mcg/ml for up to 24 hours (Figure 13).

There was no pain or other adverse effects associated with the injection of the newly synthesized DBED salt.

A paired T-test was performed with a computer program (SAS Institute) to determine if there was a statistical difference between the elimination rates after an IM administration of sodium cefazolin and an IV administration of the same formulation. The differences were statistically significant with p values of 0.0082 and 0.0068 for horses and cattle respectively. Paired T-tests were also used to compare the differences between clearance rates for sodium cefazolin administered IV vs IM. The systemic clearance (Cl_s) was not different, however, with p values of 0.273 and 0.368 for horses and cattle, respectively.

Discussion and Conclusions:

The results of these studies indicate that we have successfully synthesized a slow-release form of a cephalosporin antibiotic. A commercially available sustained release form of a cephalosporin is not currently available. DBED-cefazolin prolongs the duration of plasma drug levels far longer than what is achieved with either IV cefazolin or IM cefazolin (see Figures 5 and 13).

Cefazolin from an IV administration of sodium cefazolin persists for only 3 to 4 hours in horses and cattle which would necessitate administration via this route at least

four times daily. The elimination is more prolonged if sodium cefazolin is administered IM and persists for up to 6 to 8 hours. The pharmacokinetic parameters we have measured are similar to those reported by Sams and Ruoff (1985) and Donecker et al (1986) for cefazolin administration to horses. In their studies they found lower peak concentrations of cefazolin, however their dose was lower at 11 mg/kg in comparison to the 20 mg/kg used in our study. There are not reports available for cefazolin administration to cattle, but comparisons of various cephalosporins in different animal species were made by Caprile (1988).

The plasma concentrations following administration of DBED-cefazolin persisted for at least 24 hours. The persistence of plasma drug levels is attributed to a slow release of cefazolin from the poorly-soluble DBED-cefazolin at the IM injection site. DBED-cefazolin remained stable in storage for several months and it did not produce pain or other adverse effects following injection. These results of these pharmacokinetic parameters characterize cefazolin as a drug that is rapidly distributed and cleared in both horses and cattle. The apparent volume of distribution is low in these animals, which suggests that cefazolin is most likely limited in its distribution to plasma water and extracelluar fluid. A low volume of distribution was also reported by other authors for cefazolin (Caprile, 1988; Sams and Ruoff, 1985). Short et al (1985) reported a low volume of distribution for a cephalosporin in horses and suggested that the inability to achieve adequate concentrations of cephalosporins in the tissues of horses was because the drug is eliminated very rapidly and there is not sufficient time to reach equilibrium with tissues. If his suggestion is true, then it is apparent that either repeated administrations of cephalosporins or a slow-release form of a cephalosporin is necessary to achieve and maintain antibacterial drug concentrations.

For susceptible organisms, the minimum inhibitory concentration (MIC) for cefaz-

olin is 2.0 mcg/ml. Values below 1.0 mcg/ml are below the MIC of all single bacterial isolates of equine origin reported by Brown et al (1986), except Staphylococcus aureus, which has an MIC of 0.1 mcg/ml. For the acute treatment of infections, it is reported that plasma levels that are 4 to 5 times the MIC are needed (Powers et al 1984; Neu 1989). Although the levels of cefazolin attained after administration of DBED-cefazolin were barely above the necessary MIC level, the preparation of DBED-cefazolin may have application for prophylactic or chronic administration. Antibiotics exert effects on bacteria at concentrations below the MIC (Atkinson and Lorian, 1984) and low, but persistent concentrations of cefazolin may have a place in the treatment of certain infections.

As shown on Tables 3 and 9, the cefazolin from IM DBED-cefazolin was only 59 and 72 percent systemically available in horses and cattle respectively in comparison to the intramuscular sodium cefazolin which was over 100 percent bioavailable in both species (Tables 2 and 8). It is not known why cefazolin was not more bioavailable from DBED-cefazolin. It is possible that the intramuscular injection continued to release cefazolin beyond 32 hours, but the levels were below the sensitivity of our assay. Systemic availability was high from the IM injection of cefazolin-Na, which tends to rule out tissue binding of the cefazolin base. A larger dose injected of DBED-cefazolin would not be expected to increase the maximum plasma concentration significantly or increase the bioavailability of the drug because this drug exhibited zero order absorption characteristics.

Impact:

At this time, the full impact of these results cannot be known. We believe that the pharmacokinetic characteristics of this new compound are very promising and offers a possibility for future development. The prolonged plasma levels of cefazolin from DBED-cefazolin persist longer than any reported cephalosporin product on the market.

Until now, no investigator has been successful in prolonging the plasma levels of a cephalosporin to this extent by altering the absorption rate of the drug or prolonging its elimination. We have successfully shown that this technique of prolonging the plasma levels of a cephalosporin antibiotic is possible. We believe that the formulation of DBED-cefazolin or application of this technology to other cephalosporin products has therapeutic importance.

We are sufficiently encouraged by these results that we have instituted a patent search on this compound and it appears that there is currently no existing patent on DBED-cefazolin. We are at this time taking steps to consider the possibility of taking out our own patent on this compound.

It is possible that the most significant implications for therapeutic use lie in the administration of this compound to cattle. It is often very difficult, labour intensive, and stressful to bovine patients to administer a medication more often than once every 24 hours. Cephalosporin antibiotics are highly effective in vitro on most pathogens isolated from cattle.

III. LIST OF PAPERS AND TALKS GIVEN:

1. Papich MG, Gupta VS, Stuart A, Townsend HGG: Pharmacokinetics of a sustained-release form of cefazolin in horses. (in preparation -- to be submitted to Can. J. Vet. Res.

2. Papich MG, Gupta VS, Stuart A, Townsend HGG: Pharmacokinetics of a sustained-release form of cefazolin in horses. (in preparation -- to be submitted to Can. J. Vet. Res.

3. Papich, MG: Recent Developments in Antibacterial Therapy. Presented at the Eastern States Veterinary Conference, Orlando, Florida, Jan. 1988.

4. Papich, MG: Recent Developments in Antibiotic Therapy. To be presented at the 1988 meeting of the Canadian Veterinary Medical Assoc., July 1988.

5. Papich MG: Antibacterial Therapy in Large Animals. Presented at the Annual Meeting of the Manitoba Veterinary Association. January 13, 1989.

6. Papich MG: Antimicrobial Therapy in Large Animals. Presented at the Annual Meeting of the Alberta Veterinary Medical Association. February 3, 1989.

6. Papich, MG: Recent Developments in Antibiotic Therapy. To be presented at the 1988 meeting of the Canadian Veterinary Medical Assoc., July 1988.

IV. STATEMENT OF EXPENDITURES:

We have arranged from the University of Saskatchewan Office of Research Accounts to supply you with a statement.

V. LIST OF PERSONNEL:

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Percentage of time involved in project: 100% for 6 months.

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VIII LIST OF TABLES:

Table 1: Pharmacokinetic Values for Horses Administered Cefazolin Sodium Intravenously.[a]

Horse Number	Weight kg	Dose mg/kg	A mcg/mL	α hr-1	T1/2α hour	B mcg/mL	β hr-1	T1/2 β hour
1	602	19.93	280.17	7.47	0.093	75.06	1.41	0.49
2	495	18.18	149.20	7.32	0.095	39.90	1.47	0.47
3	423	18.90	119.55	4.58	0.150	48.76	1.30	0.53
4	422	18.96	121.97	4.97	0.139	25.59	0.97	0.71
5	461	20.00	228.49	6.24	0.111	41.45	1.04	0.67
6[b]	515	19.22	21.42	3.02	0.230	21.94	0.56	1.25
Mean	486.33	19.20	179.88	6.12	0.113[c]	46.15	1.24	0.56[b]
StdD[d]	67.92	0.69	71.34	1.32		18.21	0.22	

[a] Abreviations for all terms are defined in the text.

[b] The distribution rate constants and intercepts (A and B) from Horse Number 6 were not used in the statistical calcutaions because these values were considered outliers.

[c] Average values for T1/2α and T1/2β represent the harmonic means.

[d] Standard Deviation

Table 1 (Continued): Pharmacokinetic Values for Horses Administered Cefazolin Sodium Intravenously.

Horse Number	AUC mg/hr/L	CL_s L/kg/hr	V_c L/kg	Vd_{AREA} L/kg	K_{21} hr^{-1}	K_{10} hr^{-1}	K_{13} hr^{-1}
1	73.53	0.271	0.056	0.192	2.69	3.91	2.27
2	44.73	0.406	0.096	0.276	2.70	3.98	2.11
3	60.00	0.315	0.112	0.242	2.25	2.65	0.98
4	47.34	0.401	0.128	0.413	1.66	2.90	1.38
5	69.94	0.286	0.074	0.275	1.84	3.53	1.91
6[a]	45.04	0.427	0.443	0.762	1.80	0.94	0.84
Mean	56.76	0.35	0.090	0.280	2.23	3.39	1.73
StdD[b]	12.93	0.07	0.03	0.08	0.48	0.60	0.54

[a] The distribution rate constants, and apparent volumes of distribution (V_c and V_{AREA}) from Horse Number 6 were not used in the statistical calcutaions because these values were considered outliers

[b] Standard Deviation

Table 2: Pharmacokinetic Values for Horses Administered Cefazolin Sodium Intramuscularly.

Horse Number	Weight kg	Dose mg/kg	B mcg/mL	β hr^{-1}	T1/2β hour	AUC mg/hr/L	Cmax mcg/mL	Tmax hr	Cl_s L/kg/hr	F percent
1	585	20.5	39.53	0.504	1.38	77.86	40.0	0.25	0.263	102.95
2	486	20.0	32.63	0.538	1.29	74.02	58.0	0.25	0.270	150.42
3	395	20.3	65.90	0.462	1.50	83.93	21.0	0.75	0.242	129.93
4	442	20.0	55.86	0.775	0.90	59.07	31.8	0.50	0.339	118.29
5	464	19.83	29.06	0.495	1.40	56.60	26.2	0.25	0.350	81.62
6	563	19.54	38.70	0.539	1.28	65.49	29.4	0.50	0.298	143.02
Mean	489.17	20.03	43.61	0.55	1.26[a]	69.46	34.40	0.42	0.29	121.04
StdD[b]	72.62	0.34	14.28	0.11		10.81	13.17	0.20	0.04	25.75

[a] Harmonic mean (0.693 ÷ βmean).

[b] Standard Deviation

Table 3: Pharmacokinetic Values for Horses Administered Cefazolin-DBED Intramuscularly.

Horse Number	Weight kg	Dose mg/kg	AUC mg/hr/L	Cmax mcg/mL	Tmax hour	Cl_s L/kg/hr	F percent
1	574	20.07	41.96	2.8	4.0	0.478	56.67
2 *	485	20.00	148.23	6.5	24.0	0.135	301.23
3	400	21.50	35.85	3.0	6.0	0.600	52.52
4	454	20.35	24.63	4.0	0.5	0.826	48.47
5	440	20.88	43.27	4.2	8.0	0.483	59.26
6	495	21.19	35.53	3.4	6.0	0.596	77.55
Mean	474.67	20.67	36.25	3.48	4.90	0.60	58.89
StdD ♭	59.31	0.62	7.38	0.61	2.84	0.14	11.20

* The values from Horse Number 2 for Cl_s, AUC, Cmax, Tmax, and F were not used in the statistical calculations because these values were considered outliers.

♭ Standard Deviation

Table 4: Time - Plasma Concentrations in Horses Given Cefazolin Intravenously.

Horse Number	Time (hours) 0.05	0.10	0.17	0.25	0.33	0.50	0.75	1.0	1.5	2.0	3.0	4.0
1	-	175.0	165.0	92.0	68.0	45.0	27.0	16.0	10.0	5.0	1.0	nd[a]
2	139.2	100.6	82.9	49.5	-	26.7	13.0	9.4	4.4	2.1	1.9	nd
3	136.8	97.0	-	78.2	67.2	43.2	23.2	13.8	7.0	3.6	2.0	0.8
4	134.4	98.6	67.7	54.7	26.7	14.2	9.8	4.8	3.6	1.5	1.5	nd
5	203.6	141.6	82.8	63.2	39.2	23.0	16.8	6.8	4.4	2.2	1.4	0.8
6[b]	41.4	36.8	32.8	27.8	21.4	16.8	11.4	9.8	7.8	4.4	2.2	nd
Mean	153.5	122.6	99.6	67.5	50.3	30.4	18.0	10.2	5.9	2.9	1.6	0.8
StdE[c]	16.7	15.5	22.1	7.8	10.3	5.9	3.2	2.1	1.2	0.6	0.2	0.0

[a] nd = not detectable

[b] The values from Horse Number 6 were not used in the statistical calculations because they were considered outliers.

[c] Standard Error of the Mean

Table 5: Time - Plasma Concentrations in Horses Given Cefazolin Intramuscularly.

Horse Number	Time (hours) 0.25	0.50	0.75	1.0	1.5	2.0	3.0	4.0	6.0	8.0	10.0
1	40.0	33.0	35.0	28.0	20.9	13.8	7.3	4.4	1.9	0.8	0.5
2	58.0	47.4	37.0	25.4	16.4	11.6	5.6	2.2	1.2	0.6	0.4
3	11.5	13.5	21.0	17.3	17.5	17.5	15.5	11.4	4.0	nd[a]	nd
4	31.2	31.8	26.6	19.6	16.4	12.6	7.2	3.4	0.4	0.4	nd
5	26.2	25.8	26.0	19.6	11.6	11.2	4.4	4.0	1.8	nd	nd
6	26.7	29.4	25.5	26.1	18.4	14.0	5.8	3.6	1.9	nd	nd
Mean	32.3	30.2	28.5	22.7	16.9	13.5	7.6	4.8	1.9	0.6	0.5
StdE[b]	6.4	4.5	2.5	1.8	1.3	0.9	1.6	1.3	0.5	0.1	0.1

[a] nd = not detectable

[b] Standard Error of the Mean

Table 6: Time - Plasma Concentrations in Horses Given Cefazolin-DBED Intramuscularly.

Horse Number	Time (hours) 0.25	0.50	0.75	1.0	1.5	2.0	3.0	4.0	6.0	8.0	10.0	12.0	24.0	30.0
1	1.2	1.2	1.4	1.4	1.4	1.6	2.0	2.8	2.6	2.2	2.0	2.0	0.6	0.6
2	3.5	4.2	5.0	5.0	4.2	4.0	4.5	5.0	5.3	5.5	5.7	6.0	6.5	nd
3	1.3	1.2	1.5	1.5	1.5	2.1	2.3	2.1	3.0	2.8	1.8	1.2	0.4	nd
4	1.2	4.0	3.4	1.6	1.4	1.6	1.6	1.4	2.0	1.4	1.0	1.0	0.2	0.4
5	2.4	1.5	3.3	2.4	2.2	3.3	3.1	3.5	3.8	4.2	2.7	2.4	nd	nd
6	1.4	1.8	2.2	2.0	2.0	2.2	3.2	3.0	3.4	3.0	2.0	2.2	nd	nd
Mean	1.8	2.3	2.8	2.3	2.1	2.5	2.8	3.0	3.4	3.2	2.5	2.5	1.9	0.5
StdE*	0.4	0.6	0.6	0.6	0.4	0.4	0.4	0.5	0.5	0.6	0.7	0.7	1.5	0.1

* Standard Error of the Mean

Table 7: Pharmacokinetic Values for Cattle Administered Cefazolin Sodium Intravenously.

Cattle Number	Weight kg	Dose mg/kg	A mcg/mL	α hr-1	T1/2 α hour	B mcg/mL	β hr-1	T1/2 β hour
7	418	20.00	190.74	7.95	0.087	104.77	1.21	0.57
8	357	20.00	116.05	3.75	0.185	27.28	0.488	1.42
9	443	20.32	195.36	5.79	0.120	58.11	1.69	0.411
10	556	19.78	240.00	5.94	0.117	64.42	1.14	0.609
11	442	18.96	114.76	5.28	0.131	68.67	1.57	0.441
12	335	20.90	125.50	4.29	0.162	118.11	1.80	0.384
Mean	425.17	19.99	163.73	5.50	0.13 [a]	73.56	1.32	0.525 [a]
StdD[b]	78.16	0.64	52.31	1.47		33.01	0.48	

[a] Half-lives for alpha and beta represent the harmonic means.

[b] Standard Deviation

Table 7 (Continued): Pharmacokinetic Values for Cattle Administered Cefazolin Sodium Intravenously.

Cattle Number	AUC mg/hr/L	CL_s L/kg/hr	V_c L/kg	Vd_{AREA} L/kg	K_{21} hr^{-1}	K_{10} hr^{-1}	K_{12} hr^{-1}
7	103.76	0.193	0.069	0.160	3.60	2.67	2.90
8	82.54	0.242	0.140	0.500	1.11	1.65	1.48
9	60.49	0.336	0.080	0.199	2.63	3.72	1.13
10	87.74	0.225	0.065	0.198	2.15	3.14	1.78
11	62.91	0.301	0.103	0.191	2.96	2.80	1.09
12	76.00	0.275	0.086	0.153	3.01	2.57	0.51
Mean	78.91	0.26	0.09	0.23	2.58	2.76	1.48
StdD*	16.20	0.05	0.03	0.13	0.86	0.68	0.81

* Standard Deviation

Table 8: Pharmacokinetic Values for Cattle Administered Cefazolin Sodium Intramuscularly.

Cattle Number	Weight kg	Dose mg/kg	B mcg/mL	β hr^{-1}	T1/2β hour	AUC mg/hr/L	Cmax mcg/mL	Tmax hr	Cl_s L/kg/hr	F percent
7	420	19.05	25.90	0.434	1.60	51.84	14.19	1.00	0.367	52.45
8	361	19.39	44.46	0.472	1.47	88.73	41.74	0.50	0.219	101.88
9	419	20.00	25.49	0.352	1.97	69.38	23.25	0.25	0.288	165.53
10	545	20.00	50.61	0.428	1.62	110.76	48.75	0.50	0.181	124.85
11	417	19.18	25.98	0.489	1.42	50.59	19.87	0.25	0.379	79.49
12	342	20.47	47.61	0.755	0.92	57.56	35.00	0.50	0.356	77.33
Mean	384.00	19.68	36.68	0.49	1.41*	71.48	30.46	0.50	0.30	100.26
StdD*	38.53	0.56	12.08	0.14		23.92	13.50	0.27	0.08	

* Standard Deviation

Table 9: Pharmacokinetic Values for Cattle Administered Cefazolin-DBED Intramuscularly.

Cattle Number	Weight kg	Dose mg/kg	AUC mg/hr/L	Cmax mcg/mL	Tmax hour	F percent
7	425	20.53	10.76	1.30	8.0	10.10
8	365	21.35	99.61	10.29	8.0	113.05
9	444	19.34	48.53	2.57	6.0	84.29
10	552	20.78	90.49	3.00	24.0	98.17
11	405	22.50	54.85	3.60	8.0	73.47
12	333	22.47	41.77	3.63	6.0	51.12
Mean	420.67	21.16	57.67	4.06	10.00	71.70
StdD*	75.95	1.22	32.81	3.17	6.93	

* Standard Deviation

Table 10: Time - Plasma Concentrations in Cattle Given Cefazolin Intravenously.

Cattle Number	Time (hours) 0.05	0.08	0.17	0.25	0.33	0.50	0.75	1.0	1.5	2.0	4.0	6.0
7	133.71	151.47	142.28	135.16	-	59.82	40.13	31.21	17.41	nd[a]	nd	nd
8	140.88	116.46	73.79	69.00	-	38.46	26.41	18.04	13.81	8.47	4.20	1.43
9	189.44	183.56	117.09	-	61.90	24.09	18.82	9.90	4.38	2.09	0.27	nd
10	137.76	169.19	152.31	109.86	90.76	45.88	26.65	31.04	8.52	5.55	0.75	nd
11	126.55	130.73	114.11	118.31	50.57	39.55	22.91	12.85	6.58	3.01	0.50	nd
12	124.74	119.81	119.91	95.76	95.61	62.64	28.17	21.88	7.64	3.19	nd	nd
Mean	142.18	145.20	119.91	105.62	74.71	45.07	27.18	20.82	9.72	4.46	1.43	1.43
StdE[b]	9.79	11.20	11.13	11.16	10.96	5.90	2.93	3.67	2.00	1.15	0.76	0.0

[a] nd = not detectable

[b] Standard Error of the Mean

Table 11: Time - Plasma Concentrations in Cattle Given Cefazolin Intramuscularly.

Cattle Number	Time (hours) 0.25	0.50	0.75	1.0	1.5	2.0	4.0	6.0	8.0	10.0	12.0
7	7.19	12.94	13.22	14.19	12.64	10.83	6.61	2.05	0.66	nd[a]	nd
8	39.75	41.74	37.21	31.51	21.16	14.90	4.84	1.98	1.11	0.51	0.37
9	23.25	22.03	20.28	18.98	14.14	12.96	5.06	3.00	1.94	0.68	nd
10	46.63	48.75	43.69	39.53	27.31	17.90	6.98	2.59	1.62	0.77	0.37
11	19.87	17.60	18.12	16.67	12.22	9.41	4.31	1.19	0.45	0.23	nd
12	34.51	35.00	29.45	24.54	13.56	8.83	2.26	0.55	0.02	nd	nd
Mean	28.53	29.68	25.03	24.24	16.84	12.47	5.01	1.89	0.97	0.55	0.37
StdE[b]	5.91	5.84	4.54	3.97	2.49	1.43	0.70	0.37	0.30	0.12	0.00

[a] nd = not detectable

[b] Standard Error of the Mean

Table 12: Time - Plasma Concentrations in Cattle Given Cefazolin-DBED Intramuscularly.

Cattle Number	Time (hours) 0.25	0.50	0.75	1.0	1.5	2.0	4.0	6.0	8.0	10.0	12.0	24.0	32.0
7[a]	0.00	0.00	0.00	0.00	0.00	0.00	0.69	1.16	1.30	1.02	0.54	0.00	0.00
8	1.58	2.28	2.89	2.79	3.35	3.83	8.45	6.92	10.29	4.61	4.04	0.81	0.00
9	0.53	0.54	0.73	0.75	0.87	1.08	2.19	2.57	2.09	1.20	1.77	1.46	0.35
10	0.47	0.70	1.31	0.71	1.01	0.95	1.32	2.24	2.34	2.13	2.14	2.99	1.49
11	0.50	0.70	1.01	1.20	1.62	2.36	3.40	3.59	3.60	3.28	2.86	0.54	0.15
12	0.80	0.79	1.20	1.79	1.69	2.28	2.93	3.63	3.00	2.23	2.27	0.22	0.00
Mean	0.78	1.00	1.43	1.45	1.71	2.10	3.66	3.79	4.26	2.65	2.62	1.20	0.66
StdE[b]	0.21	0.32	0.38	0.39	0.44	0.52	1.25	0.83	1.53	0.61	0.40	0.49	0.42

[a] The values for the plasma concentrations in Cattle Number 7 were considered to be outliers and therefo were not used in the statistical calculations.

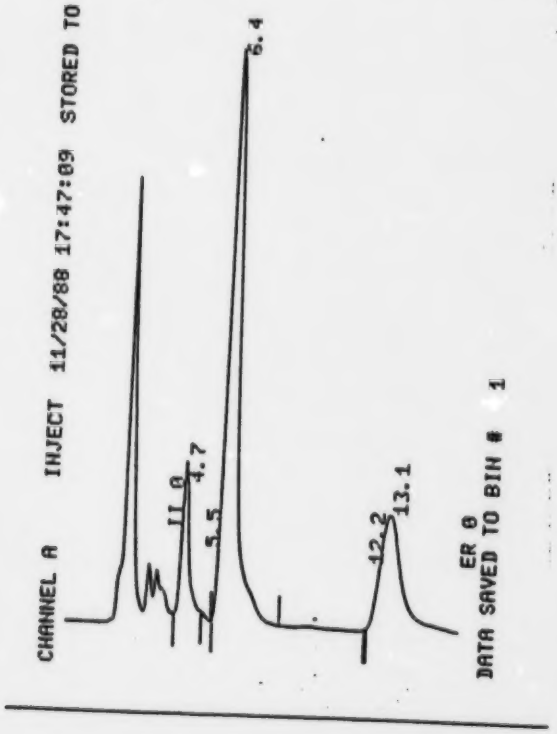
[b] Standard Error of the Mean

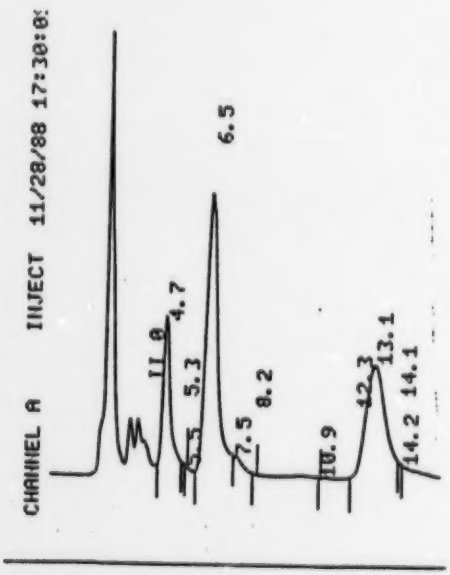
IX LIST OF FIGURES:

Figure 1: Chromatogram for Separation of Cefazolin and Cefamandole:

A sample chromatogram for the separation of cefazolin and the internal standard, cefamandole in horse plasma. The retention times for cefazolin is 6.5 min. and cefamandole is 13.0 to 13.3 min. Cefazolin was detected at a wavelength of 272 nm.

The chromatogram on the left represents the peak from cefazolin at a concentration of 50 mcg/ml and the chromatogram on the right represents cefazolin at a concentration of 25 mcg/ml. The retention time for cefazolin in both figures is 6.4 - 6.5 minutes.





FILE 1. METHOD 8. RUN 7

PEAK#	HT%	RT	PK HT BC
1	18.089	4.7	89541 02
2	1.037	5.5	5135 03
3	67.113	6.4	332221 01
4	0.141	12.2	696 02
5	13.62	13.1	67421 03
TOTAL	100.		

PEAK#	HT%	RT	PK HT BC
1	24.748	4.7	87016 02
2	0.986	5.3	3467 02
3	0.433	5.5	1523 03
4	45.808	6.5	161065 02
5	3.2	7.5	11251 02
6	0.375	8.2	1319 03
7	0.188	10.9	661 01
8	0.522	12.3	1835 02
9	18.713	13.1	65797 02
10	2.835	14.1	9968 02
11	2.193	14.2	7709 03
TOTAL	100.		351611

Figure 2: Standard Curves for Cefazolin Concentrations in Water and Plasma:

A: Standard curves for cefazolin in water (x), canine plasma (■), and equine plasma ([]) at concentrations from 0.0 to 50 ug/ml. Y-axis represents the cefazolin/internal standard peak height ratio.

B: Standard curves for cefazolin in water (x), canine plasma (■), and equine plasma ([]) at concentrations from 0.0 to 300 ug/ml. Y-axis represents the cefazolin/internal standard peak height ratio.

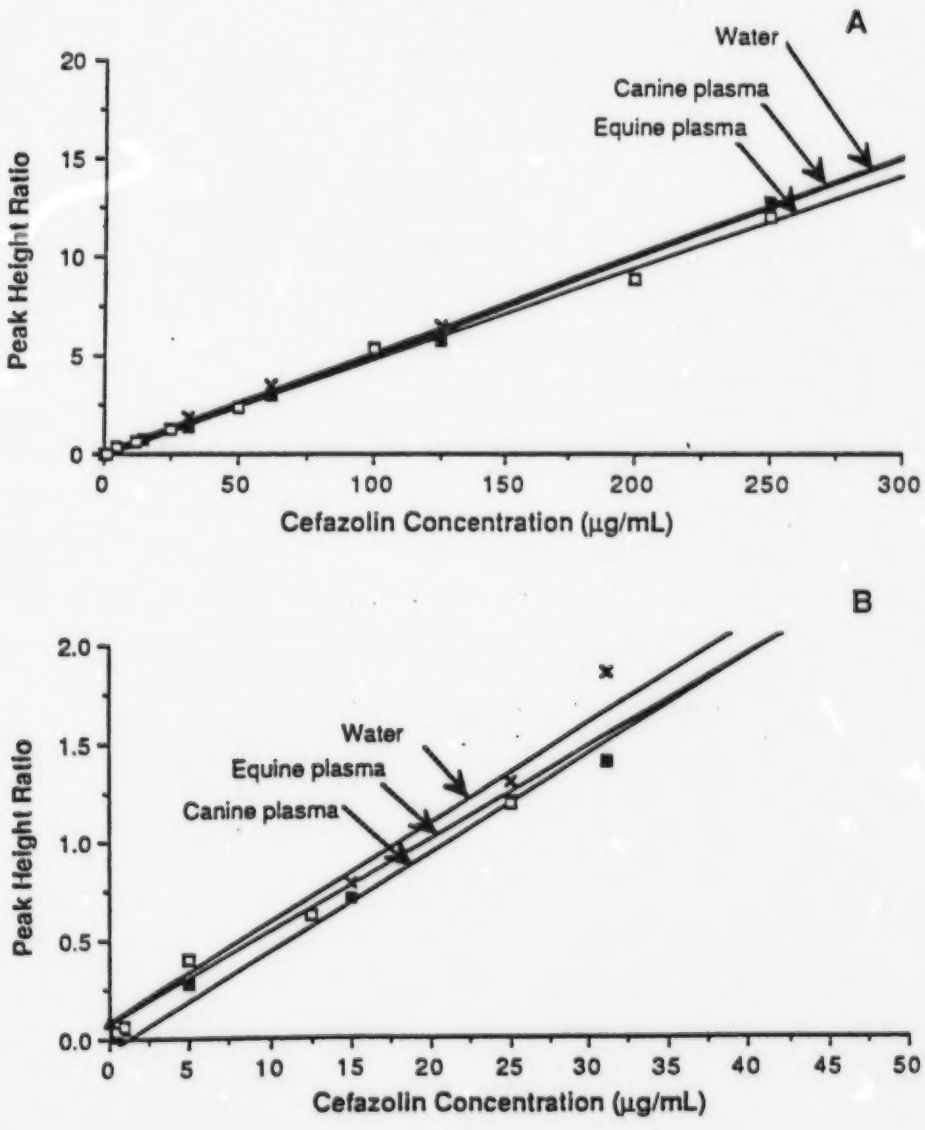
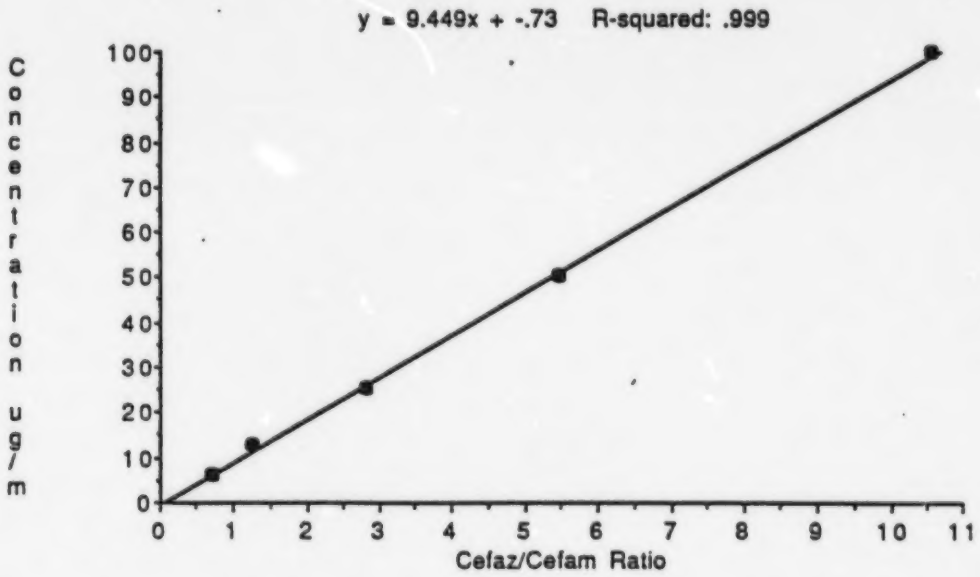


Figure 3: Standard Curve for Cefazolin Concentrations in Bovine Plasma.



Simple - Y : Concentration ug/ml X : Cefaz/Cefam Ratio

DF:	R-squared:	Std. Err.:	Coef. Var.:
4	.999	1.224	3.159

Beta Coefficient Table

Parameter:	Value:	Std. Err.:	Variance:	T-Value:
INTERCEPT	-.73	.838	.702	-.871
SLOPE	9.449	.152	.023	62.265

Analysis of Variance Table

Source	DF:	Sum Squares:	Mean Square:	F-test:
REGRESSION	1	5808.006	5808.006	3876.953
RESIDUAL	3	4.494	1.498	$p \leq .0001$
TOTAL	4	5812.5		

Residual Information Table

SS[e(i)-e(i-1)]:	e ≥ 0:	e < 0:	DW test:
9.387	3	2	2.089

Corr. Coeff. X : Cefaz/Cefam Ratio Y : Concentration ug/ml

Count:	Covariance:	Correlation:	R-squared:
5	153.674	1	.999

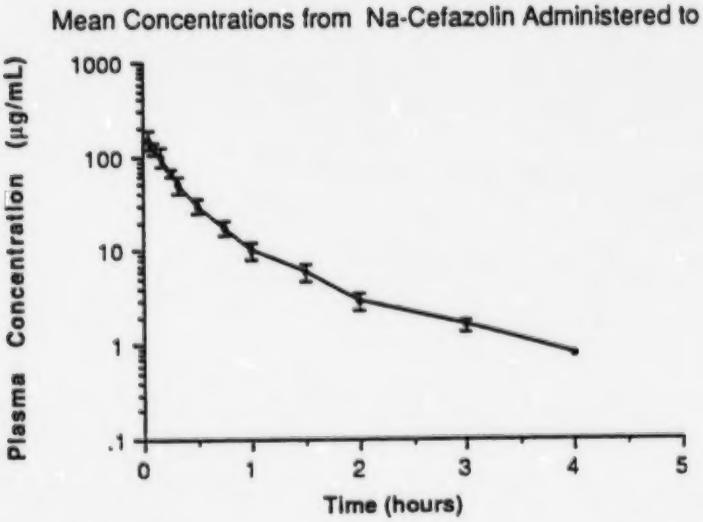


Figure 4: Mean Concentrations for Sodium Cefazolin Administered to Horses at a Mean Dose of 19.2 mg/kg.

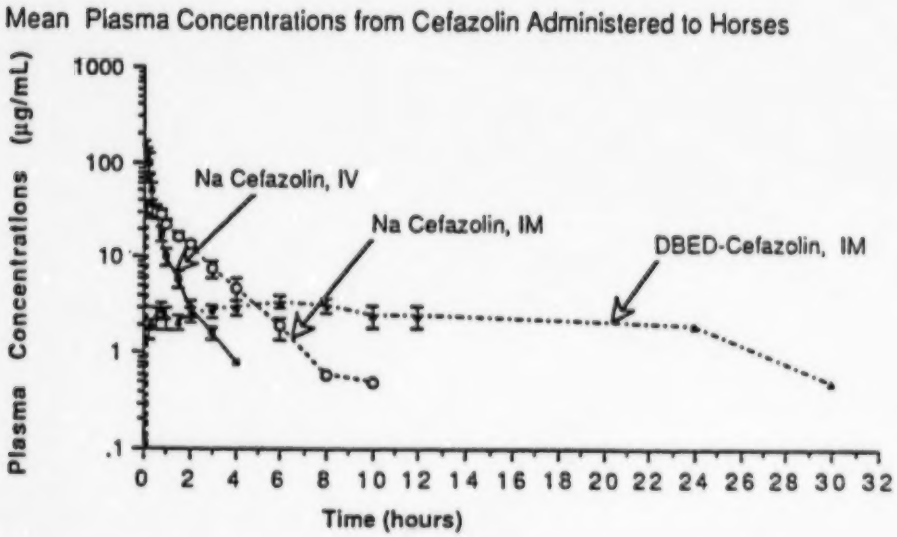


Figure 5: Mean Plasma Concentrations from Cefazolin Administered to Horses as Sodium Cefazolin and DBED-cefazolin. (Vertical bars represent ± 1 Standard Error of the Mean).

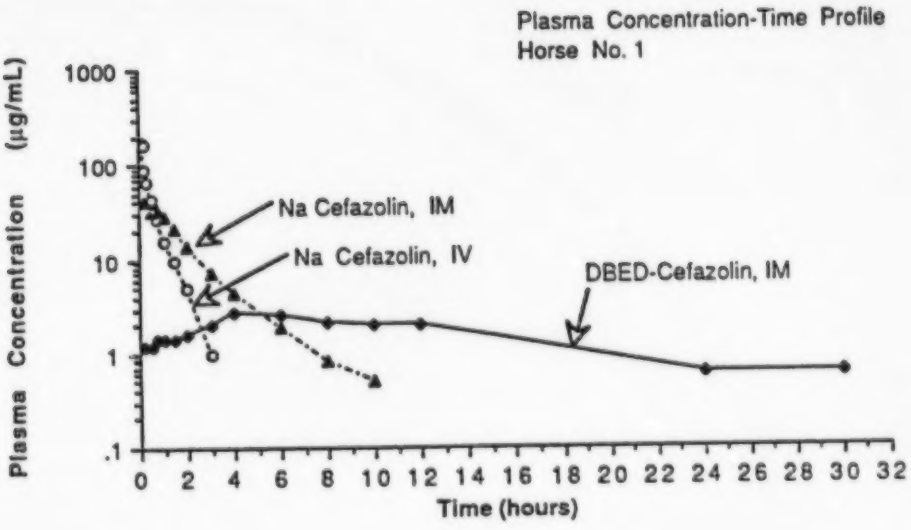


Figure 6: Plasma Concentration-Time Profile from Cefazolin Administered to Horse Number 1.

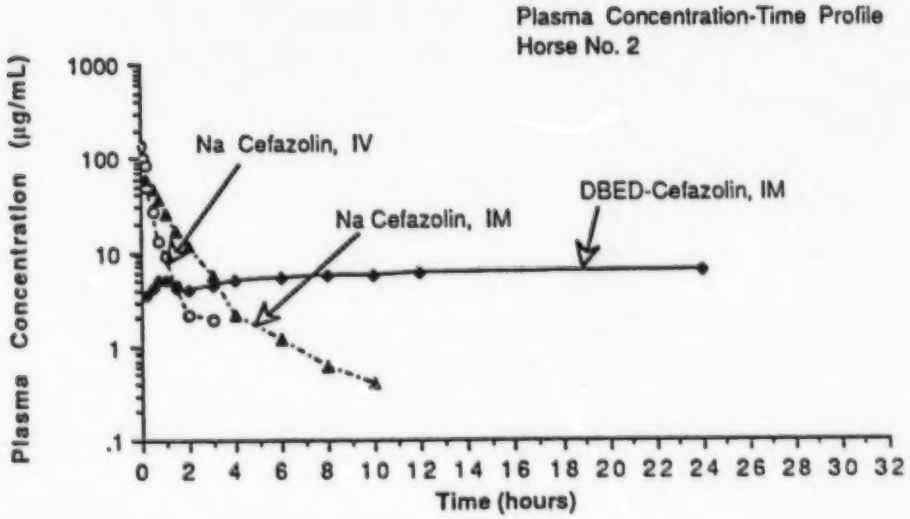


Figure 7: Plasma Concentration-Time Profile from Cefazolin Administered to Horse Number 2.

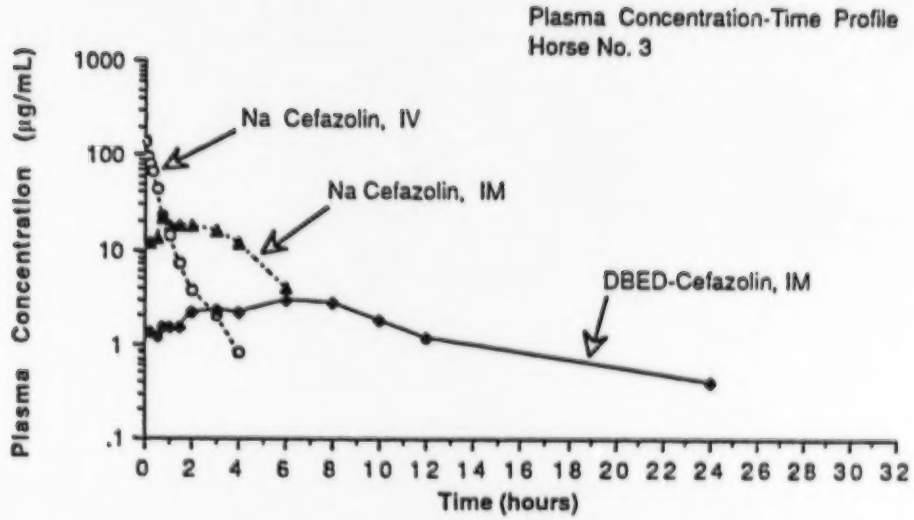


Figure 8: Plasma Concentration-Time Profile from Cefazolin Administered to Horse Number 3.

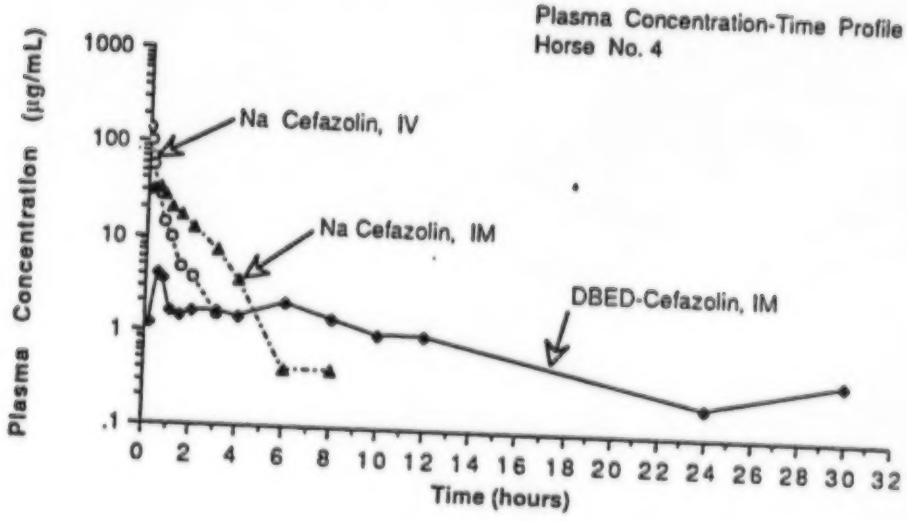


Figure 9: Plasma Concentration-Time Profile from Cefazolin Administered to Horse Number 4.

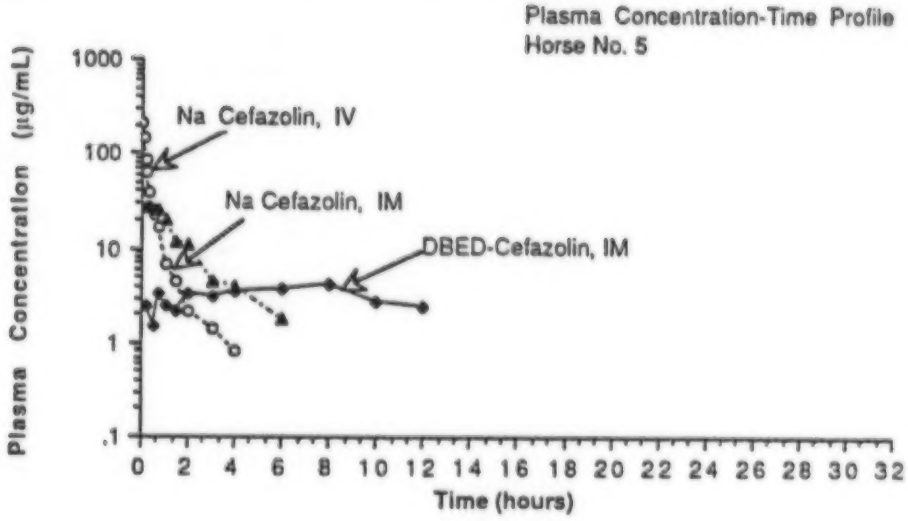


Figure 10: Plasma Concentration-Time Profile from Cefazolin Administered to Horse Number 5.

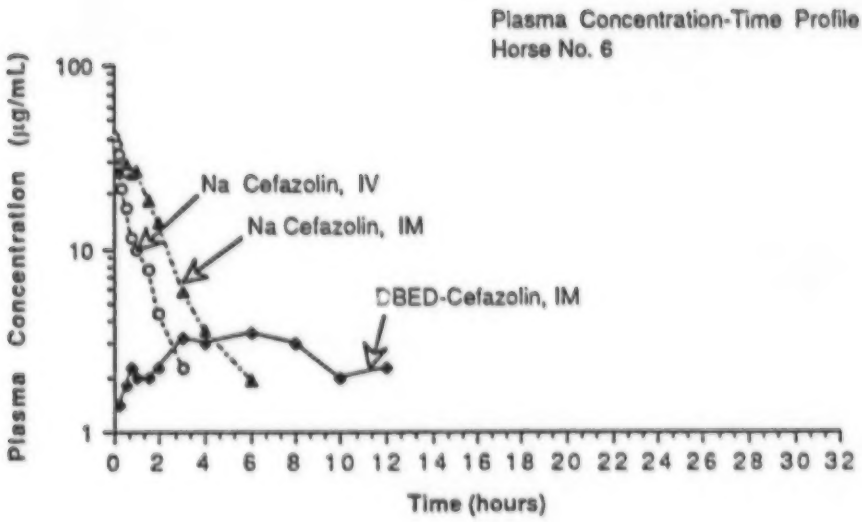


Figure 11: Plasma Concentration-Time Profile from Cefazolin Administered to Horse Number 6.

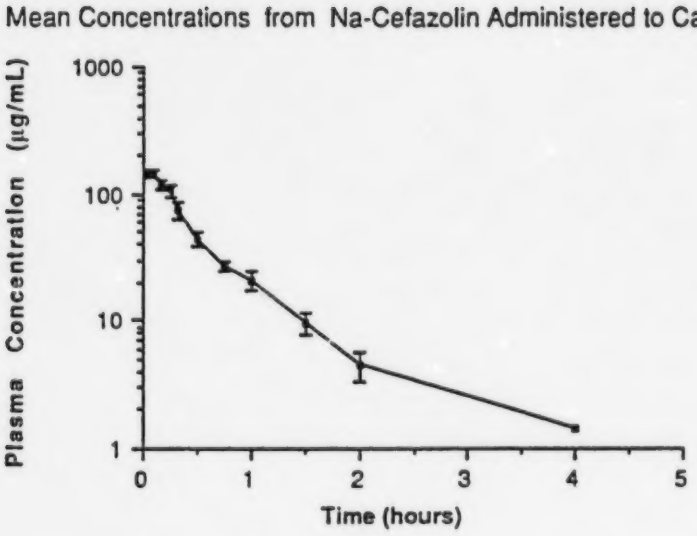


Figure 12: Mean Concentrations from Sodium Cefazolin Administered to Cattle, IV at a dose of 19.99 mg/kg.

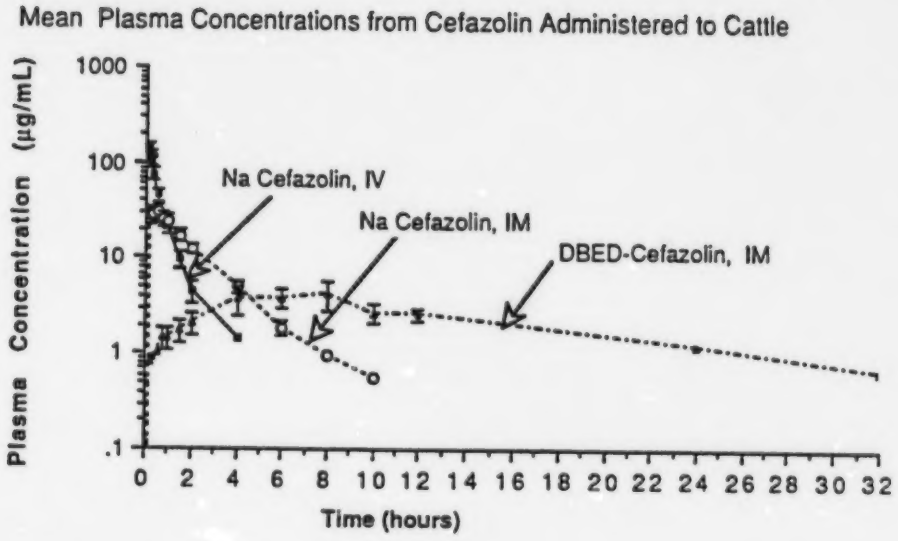


Figure 13: Mean Plasma Concentrations from Cefazolin Administered to Cattle as Sodium Cefazolin and DBED-cefazolin. (Vertical bars represent ± 1 Standard Error of the Mean).

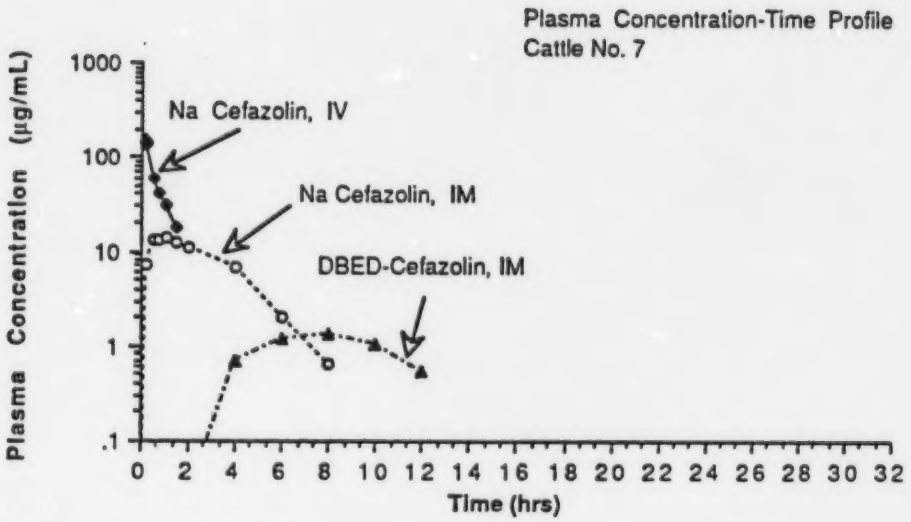


Figure 14: Plasma Concentration-Time Profile from Cefazolin Administered to Cattle: Animal Number 7.

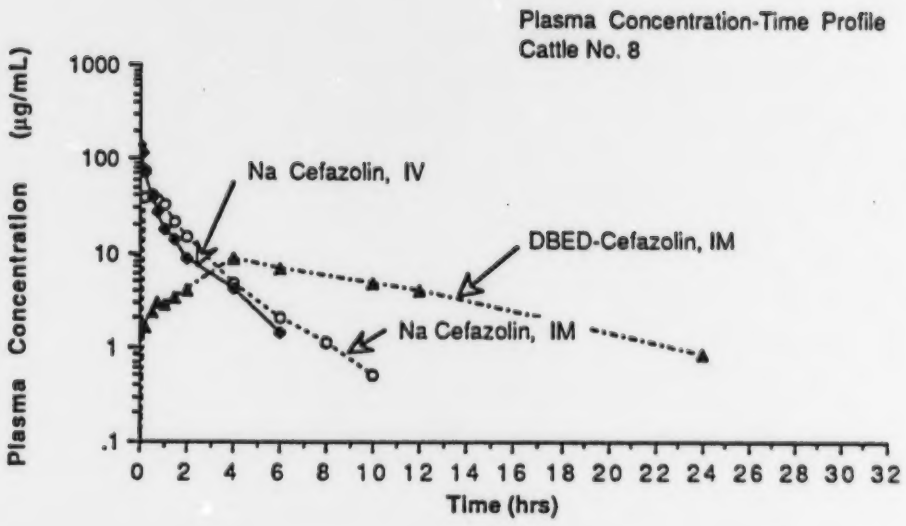


Figure 15: Plasma Concentration-Time Profile from Cefazolin Administered to Cattle: Animal Number 8.

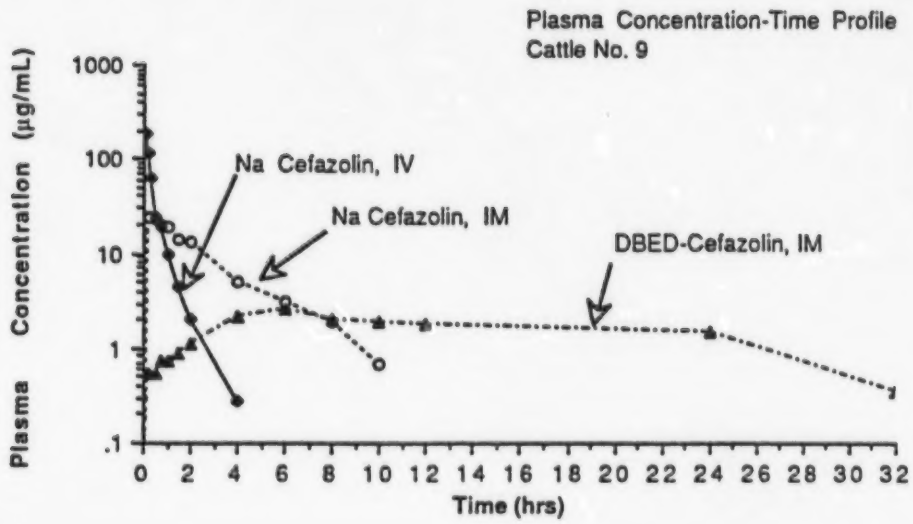


Figure 16: Plasma Concentration-Time Profile from Cefazolin Administered to Cattle: Animal Number 9.

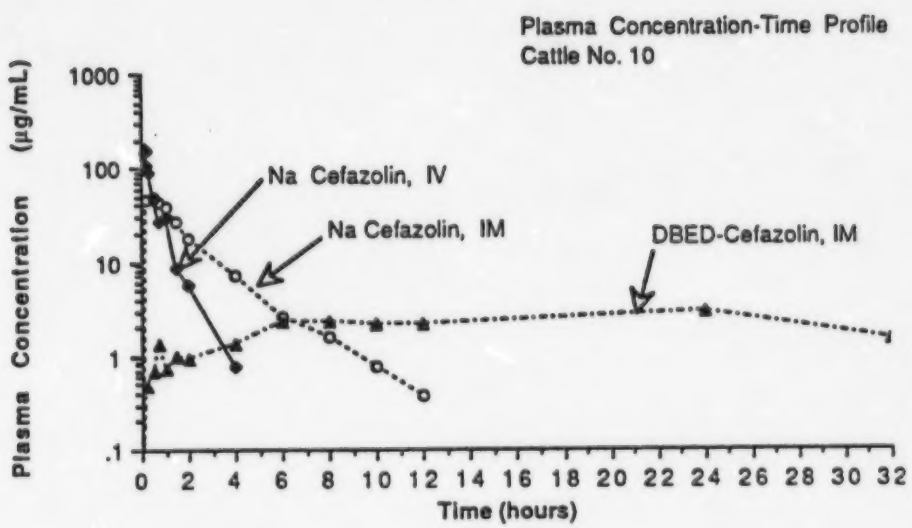


Figure 17: Plasma Concentration-Time Profile from Cefazolin Administered to Cattle: Animal Number 10.

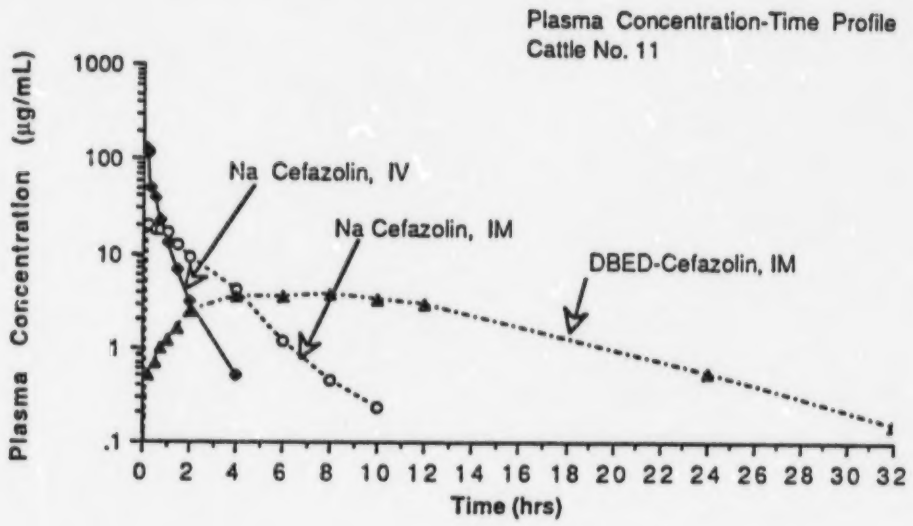


Figure 18: Plasma Concentration-Time Profile from Cefazolin Administered to Cattle: Animal Number 11.

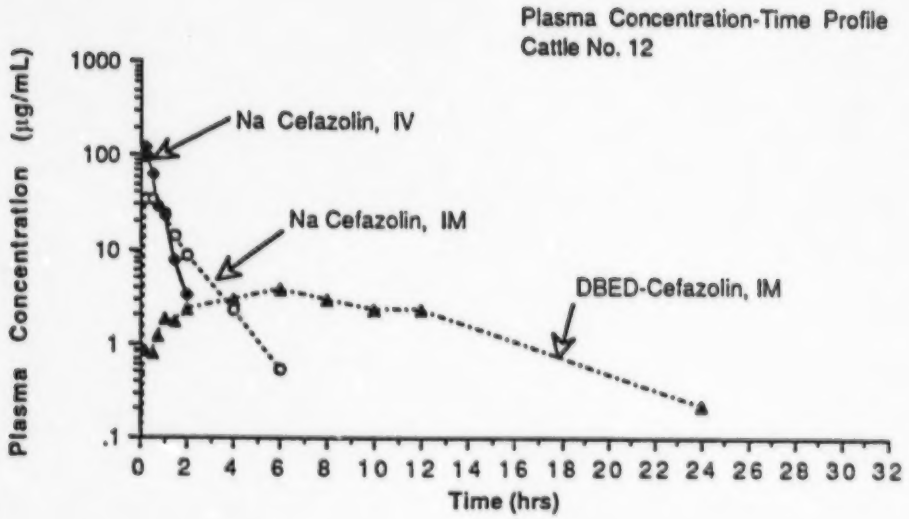


Figure 19: Plasma Concentration-Time Profile from Cefazolin Administered to Cattle: Animal Number 12.